Prediagnostic toenail selenium and risk of bladder cancer

Prediagnostic Toenail Selenium and Risk of Bladder Cancer[1]

Maurice P. A. Zeegers,[2] R. Alexandra Goldbohm, Peter Bode, and Piet. A. van den Brandt

Department of Epidemiology, Maastricht University, 6200 MD Maastricht, the Netherlands [M. P. A. Z., P. A. v. d. B.]; Department of Nutritional Epidemiology, TNO Nutrition and Food Research, 3700 AJ Zeist, the Netherlands [R. A. G.]; and Interfaculty Reactor Institute, Delft University of Technology, 2629 JB Delft, the Netherlands [P. B.]

Abstract

The association between several cancers and selenium status has been investigated in epidemiological studies. However, few results concerning bladder cancer have been reported thus far. The association between toenail selenium status and subsequent bladder cancer incidence was investigated in a prospective cohort study among 120,852 men and women aged 55–69 years at baseline (September 1986). The cohort members completed a questionnaire on risk factors for cancer and provided toenail clippings for determination of baseline selenium status. Follow-up for incident cancer was established by record linkage to cancer registries until December 1992. The multivariable case-cohort analysis was based on 431 bladder cancer cases and 2,459 subcohort members, for whom toenail selenium levels were available. The age-, sex- and smoking-adjusted rate ratios (95% confidence intervals) for increasing quintiles of toenail selenium were 1.00 (reference), 1.09 (0.80–1.48), 0.55 (0.38–0.79), 0.63 (0.43–0.91), and 0.67 (0.46–0.97), respectively (*P*-trend < 0.01). Analyses with selenium as a continuous variable supported these findings. An inverse association between toenail selenium and bladder cancer risk was most pronounced among ex-smokers (*P*-trend < 0.01); was similar for subjects with high *versus* low intakes of β-carotene, vitamin C, and vitamin E; and was mainly confined to invasive transitional cell carcinomas of the urinary bladder, irrespective of tumor morphology. We conclude that the evidence is in favor of an inverse association between selenium and bladder cancer risk.

Introduction

Bladder cancer is the most common cancer of the urinary tract and is the seventh most common cancer among men, accounting for approximately 200,000 new cases per year worldwide (1). Over the last four decades, many epidemiological studies and several reviews have been conducted to investigate determinants of bladder cancer (2–6). These studies suggested that bladder cancer is influenced by environmental factors, including cigarette smoking, fluid consumption, schistosomal infections, exposure to industrial chemicals (*e.g.*, aromatic amines), and diet.

It has been suggested that selenium may protect against cancer, particularly because of its antioxidant properties, although other mechanisms have also been proposed (7–9). Selenium is an essential trace element required for normal function, growth, and reproduction (10, 11). Several animal, ecological, and epidemiological studies support the hypothesis that low intake of selenium is associated with an increased risk of cancer (8). However, only five epidemiological studies have concentrated on urinary bladder cancer incidence (7–9, 12, 13). Most of these studies assessed short-term selenium intake in serum and were based on small numbers of cases.

In the present study, we were able to explore the relationship of prediagnostic toenail selenium to human bladder cancer prospectively with substantially more cases than in previous studies.

Materials and Methods

Cohort. The Netherlands Cohort Study is a population-based prospective study on diet and cancer started in the Netherlands in September 1986. The cohort includes 58,279 men and 62,573 women aged 55–69 years at the start of the study. The study population originated from 204 municipal population registries throughout the country. The case cohort approach was used for data processing and analysis (14). Cases were enumerated from the entire cohort, whereas the accumulated person years in the cohort were estimated from a subcohort sample. Following this approach, a subcohort of 3,500 subjects (1,688 men and 1,812 women) was randomly sampled from the cohort after the baseline exposure measurement. The subcohort has been followed up for vital status information. No subcohort members were lost to follow-up, during the follow-up period. The study design, including data collection strategies, has been described in detail previously (15).

Follow-Up. Follow-up for incident cancer was established by record linkage to cancer registries and the Dutch national database of pathology reports (16). The completeness of cancer follow-up was estimated to be over 96% (17). The present analysis is restricted to cancer incidence in 6.3 years of follow-up, from September 1986 to December 1992, because only information of toenail selenium status for cases diagnosed until 1992 were available at the time of the analysis. After excluding prevalent cases with cancer other than skin cancer, a total of 3,346 subcohort members (1,630 men and 1,716 women) and 619 incident cases (532 men and 87 women) with microscopically confirmed, incident carcinomas of the urinary bladder, ureters, renal pelvis, or urethra were identified. Of these cases, 584 (94.3%) were diagnosed with bladder cancer, of which 559 (95.7%) were transitional cell carcinomas. Because the overwhelming majority of tumors occurred in the urinary bladder

Received 1/18/02; revised 6/5/02; accepted 6/15/02.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] The Netherlands Cohort Study was supported by the Dutch Cancer Society.
[2] To whom requests for reprints should be addressed, at Department of Epidemiology, Maastricht University, P. O. Box 616, 6200 MD Maastricht, the Netherlands. Phone: 31-43-3882371; Fax: 31-43-3884128; E-mail: mpa.zeegers@epid.unimaas.nl.

and the ureters, renal pelvis, and urethra are covered by the same urothelium as the urinary bladder, the term bladder cancer is used as a synonym for these neoplasms.

Exposure Status. At baseline, all cohort members completed a self-administered questionnaire on risk factors for cancer and potential confounding variables. The questionnaire data were key entered twice and processed in a standardized manner, blinded with respect to case/subcohort status to minimize observer bias in coding and interpretation of the data. Toenail clippings had been provided by 438 patients with bladder cancer (76.8%) and by 2569 (70.8%) subcohort members.

In 1992, the toenail selenium analyses for subcohort members were carried out by the Interfaculty Reactor Institute at Delft University (Delft, the Netherlands), using instrumental neutron activation analysis. This method, using the SBP facility, and evaluation of accuracy and precision have been described elsewhere (18).

In 1996, toenail selenium levels of bladder cancer cases were determined with the CAFIA, also carried out by the Interfaculty Reactor Institute (19). The selenium determination for the cases was also based on the measurement of the induced radioactivity of the short-lived 77mSe radionuclide (half-life, 17.5 s), but cyclic activation and measurement was applied to achieve the required sensitivity (20). Each sample passed 6 successive cycles of 17-s irradiation at a thermal neutron flux of 3.7.1016 m-2s-1, 3 s decay, and 17 s counting at 1 cm from a 40% Ge detector. The results of the six countings were added. The accuracy of the instrumental neutron activation analysis was checked by analysis of a certified bovine liver standard (Standard Reference Material 1577b of the United States National Institute of Standards and Technology). For 30 determinations, a mean value ± SD of 0.74 ± 0.04 μg/g selenium ($N = 49$) was observed against a certified value of 0.73 ± 0.06 μg/g.

Because of problems with the detection of selenium in samples weighing less than 10 mg and sometimes in samples with very high calcium contents, 7 and 110 specimens were excluded from the bladder cancer and subcohort groups, respectively. Together with the case group, the toenail selenium levels for 40 subcohort members were assessed with the CAFIA facility in addition to original assessment with the SBP facility to evaluate comparability. The mean selenium level ± SD using the SBP facility (0.551 ± 0.04 μg/g) was comparable to mean selenium levels using the CAFIA facility (0.552 ± 0.05 μg/g) for these subjects. The Pearson correlation coefficient between toenail selenium levels assessed by the CAFIA facility and those estimated by the SBP facility was 0.95 ($P < 0.01$). Toenail selenium data were available for analysis from 431 bladder cancer cases (372 men and 59 women) and 2459 subcohort members (1211 men and 1248 women).

Statistical Analyses. To evaluate the potential influence of prediagnostic cancer on toenail selenium levels, we categorized the cases according to the year of follow-up in which the diagnosis was made. Mean toenail selenium levels of bladder cancer cases were compared according to year of follow-up. Toenail selenium levels were categorized into quintiles according to the distribution in the subcohort. Incidence RRs[3] and corresponding 95% CIs for bladder cancer were estimated using exponentially distributed failure time regression models (21) with the Stata statistical software package (22). Standard errors were estimated using the robust Hubert-White sandwich estimator to account for additional variance introduced by sampling from the cohort. This method is equivalent to the variance-covariance estimator presented by Barlow (23) and Lin and Ying (24). Because of skewedness to the right, continuous data on toenail selenium concentrations were normalized with a $\log_e$ transformation. The RR was calculated per standard unit (z-score) of toenail selenium levels. This is comparable to an increment of 0.202 μg/g selenium. We confirmed constancy of the baseline hazard visually by plotting the natural logarithm of the baseline survival function against failure time.

The following variables were considered as potential confounders: intake of alcohol (g/day), coffee (ml/day), tea (ml/day), water (ml/day), vegetables (g/day), and fruit (g/day); current cigarette smoking (yes/no); smoking amount (cigarettes/day); smoking duration (years of cigarette smoking); occupational exposure to dye, rubber, leather, or vehicle fumes (ever/never); and first-degree family history of bladder cancer (yes/no). Those variables that showed a more than 10% influence on the risk of bladder cancer when considered in a multivariable model were included as covariates in multivariable analyses. If one or more dietary covariates were incorporated in the regression model, subjects with incomplete or inconsistent dietary data were excluded, according to criteria described previously (25). Subgroup analyses conditional on cigarette smoking status (never/ex/current) and years since smoking cessation were performed to evaluate interaction of selenium by smoking. Because earlier studies (13, 26, 27) suggested a potential interaction between vitamin levels and selenium, we stratified the results by high *versus* low β-carotene, vitamin C, and vitamin E intake status of the subjects. Low and high are defined as the two lowest quintiles and the two highest quintiles of intake, respectively. Furthermore, subgroup analyses were conducted after stratification by morphology and invasiveness of bladder cancer. Because the RRs concerning toenail selenium level and bladder cancer risk were similar for men and women, the results are presented for men and women combined.

Results

Table 1 displays the average toenail selenium levels among bladder cancer cases according to sex and year of follow-up. Those of the subcohort are presented in a footnote of the table. Female cases appeared to have higher toenail selenium levels than male cases ($P = 0.02$). The mean toenail selenium level for the cases (men, 0.536 μg/g; women, 0.568 μg/g) was lower than that for the subcohort members (men, 0.547 μg/g; women, 0.575 μg/g). Cases diagnosed in the first years of follow-up had lower toenail selenium levels than cases diagnosed later after adjustment for sex (Table 1).

Toenail selenium was inversely associated with bladder cancer risk in men and women combined (Table 2). The age- and sex-adjusted RR was 0.54 (95% CI, 0.38–0.76) comparing highest to lowest quintiles of toenail selenium. After adjustment for age, sex, number of cigarettes smoked per day, and years of cigarette smoking, the RR was 0.67 (95% CI 0.46–0.97) comparing the same quintiles of toenail selenium (P-trend < 0.01). This reduced bladder cancer incidence appears to be limited to the highest three quintiles of the toenail selenium distribution. The RR decreased by 7% for each increment of 1 standard unit of toenail selenium (0.202 μg/g). After exclusion of those patients whose cancer was diagnosed during their first year of follow-up, the negative association between toenail selenium

[3] The abbreviations used are: RR, rate ratio; CI, confidence interval; SBP, Snelle Buizenpost; CAFIA, Carbonfiber Autonomous Facility for Irradiation and Analysis.

Table 1 Toenail selenium levels (μg/g) in bladder cancer cases according to sex and time between baseline and bladder cancer diagnosis; Netherlands Cohort Study (1986–1992)

Cases[a]	No. of subjects	Toenail selenium level (μg/g)		
		Mean	SD	P^b
Sex				
Men	372	0.536	0.215	Reference
Women	59	0.568	0.122	0.02
Year of follow-up[c]				
1	47	0.518	0.091	0.04
2	61	0.523	0.263	0.21
3	74	0.546	0.128	0.50
4	75	0.544	0.155	0.90
5	70	0.568	0.154	0.63
6	84	0.590	0.721	Reference
7	20	0.595	0.123	Reference

[a] Mean ± SD selenium levels in subcohort members were 0.547 ± 0.126 μg/g for men (n = 1211) and 0.575 ± 0.109 μg/g for women (n = 1248).
[b] Partial t tests between strata and reference group, based on In-transformed toenail selenium levels.
[c] Presented results per year of follow-up were adjusted for sex through linear regression analysis.

Table 2 Incidence RR of bladder cancer according to toenail selenium levels; Netherlands Cohort Study (1986–1992)

Quintiles of toenail selenium (boundaries in μg/g)	All years of follow-up				First year excluded
	Cases in cohort	Person years in subcohort	RR (95% CI)[a]	RR (95% CI)[b]	RR (95% CI)[b]
1 (≤0.483)	114	2986	1.00 (reference)	1.00 (reference)	1.00 (reference)
2 (0.483 to ≤0.530)	116	3022	1.05 (0.79–1.40)	1.09 (0.80–1.48)	1.19 (0.86–1.64)
3 (0.530 to ≤0.573)	78	3031	0.51 (0.36–0.73)	0.55 (0.38–0.79)	0.61 (0.42–0.89)
4 (0.573 to ≤0.630)	62	2993	0.53 (0.37–0.76)	0.63 (0.43–0.91)	0.63 (0.42–0.94)
5 (>0.630)	61	3001	0.54 (0.38–0.76)	0.67 (0.46–0.97)	0.78 (0.54–1.14)
P for linear trend			<0.01	<0.01	<0.01
Increment in standard units[c]			0.86 (0.77–0.96)	0.94 (0.83–1.05)	0.89 (0.79–1.00)

[a] Adjusted for age (years) and sex.
[b] Adjusted for age, sex, number of cigarettes/day, and years of cigarette smoking.
[c] z-scores based on In-transformed toenail selenium levels (increment of 0.202 μg/g).

Table 3 Incidence RR of bladder cancer according to toenail selenium levels and smoking status; Netherlands Cohort Study (1986–1992)

Smoking status and cessation[a]	No. of cases	Quintile of toenail selenium (boundaries in μg/g)					P trend
		1 (≤0.483)	2 (≤0.530)	3 (≤0.573)	4 (≤0.630)	5 (>0.630)	
Never[b]	51	1.00 (reference)	2.69 (1.02–7.09)	1.37 (0.50–3.79)	1.09 (0.38–3.14)	1.36 (0.50–3.69)	0.62
Ex[c]	186	1.00 (reference)	0.74 (0.46–1.19)	0.48 (0.28–0.81)	0.49 (0.29–0.83)	0.44 (0.26–0.75)	<0.01
≤10 yrs	89	1.00 (reference)	0.42 (0.19–0.94)	0.47 (0.21–1.08)	0.45 (0.20–1.01)	0.30 (0.12–0.75)	<0.01
>10 yrs	96	1.00 (reference)	1.06 (0.55–2.06)	0.48 (0.22–1.01)	0.47 (0.20–1.09)	0.58 (0.29–1.18)	<0.01
Current[c]	170	1.00 (reference)	1.36 (0.86–2.14)	0.45 (0.24–0.86)	0.76 (0.39–1.47)	1.13 (0.56–2.27)	0.25

[a] P for interaction was 0.19.
[b] Adjusted for age (years) and sex.
[c] Adjusted for age (years), sex, number of cigarettes/day, and years of cigarette smoking.

level and bladder cancer risk persisted (Table 2). Additional correction for current cigarette smoking, coffee, tea, water, vegetables, fruit, occupational exposure, and family history of bladder cancer did not change the risk substantially (data not shown).

We did not find statistically significant interactions regarding bladder cancer risk between toenail selenium and cigarette smoking status (P = 0.19), amount (P = 0.76), or duration (P = 0.61). However, an inverse association between toenail selenium and bladder cancer risk was limited to ex-smokers (P-trend < 0.01), and no inverse association was found among never and current smokers (Table 3). The correlation between toenail selenium status and years of smoking cessation was 0.08 (P < 0.01). Among ex-smokers, the association between toenail selenium and bladder cancer risk was comparable for those who have refrained from smoking for >10 years and those who have refrained from smoking for ≤10 years (Table 3).

To evaluate effect modification of the association between toenail selenium and bladder cancer risk by the intake level of antioxidant vitamins β-carotene, vitamin C, and vitamin E, we also evaluated whether the association with bladder cancer depended on intake of these antioxidants (Table 4). The inverse association between toenail selenium and bladder cancer risk persisted for subjects with different intakes of β-carotene, vitamin C, and vitamin E (Table 4). We found no statistically

Table 4 Incidence RR[a] of bladder cancer according to toenail selenium levels by category of vitamin intake; Netherlands Cohort Study (1986–1992)

Group	No. of cases	Quintile of toenail selenium (boundaries in μg/g)					*P*-trend
		1 (≤0.483)	2 (≤0.530)	3 (≤0.573)	4 (0.630)	5 (>0.630)	
β-Carotene intake[b]							
Low	167	1.00 (reference)	0.98 (0.59–1.62)	0.50 (0.28–0.89)	0.71 (0.40–1.26)	0.65 (0.36–1.16)	0.02
High	155	1.00 (reference)	1.08 (0.65–1.80)	0.97 (0.55–1.71)	0.66 (0.34–1.26)	0.74 (0.41–1.34)	0.09
Vitamin C intake[b]							
Low	181	1.00 (reference)	0.95 (0.59–1.53)	0.45 (0.26–0.78)	0.72 (0.41–1.26)	0.77 (0.43–1.37)	0.05
High	140	1.00 (reference)	1.09 (0.64–1.85)	1.01 (0.57–1.80)	0.50 (0.25–1.00)	0.75 (0.41–1.37)	0.06
Vitamin E intake[b]							
Low	145	1.00 (reference)	1.37 (0.81–2.33)	0.58 (0.30–1.09)	0.76 (0.41–1.42)	0.82 (0.45–1.50)	0.07
High	170	1.00 (reference)	1.09 (0.68–1.75)	0.59 (0.34–1.03)	0.70 (0.38–1.28)	0.73 (0.41–1.29)	0.04

[a] Adjusted for age (years), sex, number of cigarettes/day, and years of cigarette smoking.
[b] Low and high are defined as the two lowest quintiles and the two highest quintiles of intake, respectively. For β-carotene, the cutoff values for low and high were: ≤2.36, >2.97 mg/day; for vitamin C the corresponding values were ≤86.60 and >107.71 mg/day, and for vitamin E the corresponding values were ≤10.72 and <13.89 mg/day. The *P*s for interaction were 0.58, 0.16, and 0.96 for β-carotene, vitamin C, and vitamin E.

Table 5 Incidence RRs for transitional cell carcinoma of the urinary bladder according to toenail selenium levels, with respect to tumor invasiveness and morphology; Netherlands Cohort Study 1986–1992

Quintiles of toenail selenium (boundaries in μg/g)	Noninvasive ($T_{IS}/T_a/T_1$)		Invasive (T_{2-4})	
	Non-papillary[a] (*n* = 16)	Papillary[b] (*n* = 180)	Non-papillary[a] (*n* = 81)	Papillary[b] (*n* = 108)
	RR (95% CI)[c]	RR (95% CI)[c]	RR (95% CI)[c]	RR (95% CI)[c]
1 (≤0.483)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
2 (0.483 to ≤0.530)	1.24 (0.31–4.88)	1.23 (0.80–1.88)	1.11 (0.63–1.95)	0.96 (0.58–1.59)
3 (0.530 to ≤0.573)	1.07 (0.25–4.58)	0.63 (0.37–1.05)	0.45 (0.21–0.96)	0.44 (0.23–0.84)
4 (0.573 to ≤0.630)	1.28 (0.26–6.42)	0.78 (0.46–1.31)	0.17 (0.05–0.57)	0.64 (0.34–1.20)
5 (>0.6300)	0.83 (0.13–5.20)	0.91 (0.55–1.50)	0.62 (0.30–1.28)	0.36 (0.17–0.75)
P for linear trend	0.92	0.18	<0.01	<0.01
Increment in standard units[d]	1.08 (0.74–1.58)	1.06 (0.87–1.29)	0.84 (0.69–1.02)	0.82 (0.72–0.94)

[a] ICD-O:M8120.
[b] ICDO:M8130.
[c] Adjusted for age, sex, number of cigarettes smoked/day, and years of cigarette smoking.
[d] z-scores based on In-transformed toenail selenium levels (increment of 0.202 μg/g).

significant interaction effect between these antioxidants and toenail selenium ($P = 0.58$, 0.16, and 0.96, respectively).

An inverse association between toenail selenium level and bladder cancer risk was most pronounced in invasive transitional cell carcinomas of the urinary bladder, irrespective of tumor morphology (*P*-trend < 0.01 and < 0.01 for invasive papillary and non-papillary tumors, respectively; Table 5). The RRs for noninvasive tumors did not indicate a clear inverse association for toenail selenium (Table 5).

Discussion

These data showed a small inverse association between baseline toenail selenium and subsequent bladder cancer risk. The association was most pronounced in ex-smokers and was mainly confined to invasive carcinomas of the bladder. The association appeared to be independent of β-carotene, vitamin C, and vitamin E intake.

Preclinical symptoms of bladder cancer might influence selenium status. However, one advantage of this and other prospective studies is that selenium status was assessed before diagnosis of bladder cancer. In the present study, cases diagnosed in the first years of follow-up had lower toenail selenium levels than cases diagnosed later. This indicates that preclinical disease may have had influence on selenium status in the first year of follow-up, although it might also be that lower selenium status in fact causes bladder cancer to happen sooner. We found a strong inverse association between continuous selenium exposure and first year bladder cancer incidence (RR, 0.18; 95% CI, 0.06–0.51). After exclusion of cases diagnosed in the first year of follow-up, however, the diluted results were similar to results based on all cases (Table 2). Selection bias is not likely because the follow-up for cancer incidence and person years was almost complete (17, 28). Nondifferential information bias might be prevalent because different selenium assays were used to assess selenium concentration in toenails. However, both CAFIA and SBP methods appeared to be highly comparable to the gold standard (bovine liver), and their correlation was very high ($r = 0.95$). We therefore expect that the small difference between both methods will not systematically bias the results. The large number of bladder cancer cases in this study was another important advantage.

Diet assessment methods are inadequate to assess selenium intake because selenium content of an individual food can vary widely due to geographic variation in soil selenium content. Therefore, previous epidemiological studies on the relation between selenium and bladder cancer have often relied on biological markers of selenium status, such as serum selenium (7, 8, 13) or toenail selenium (12). A selenium marker should preferably reflect long-term selenium intake. For this reason, we used levels of selenium in toenails to assess selenium intake instead of serum selenium, a short-term marker of selenium intake (18). Available evidence suggests that selenium levels in

Table 6 Prospective studies of selenium in relation to urinary tract cancer incidence

Study	Country	Sex	Cases	Mean selenium level (cases/controls)	Exposure	Comparison	Site	RR (95% CI)
Nomura *et al.*, 1987 (13)	US	Men	29	12.11/12.49 μg/dl	Serum	Highest *vs.* lowest quintile	Bladder	0.32
Helzlsouer *et al.*, 1989 (7)	US	Both	35	11.1/11.7 μg/dl	Serum	Highest *vs.* lowest tertile	Bladder	0.49 (0.16–1.49)
Knekt *et al.*, 1990 (8)	Finland	Men	26	6.16/6.53 μg/dl	Serum	Four higher *vs.* lowest quintile	Urinary tract	0.34 (0.06–2.06)
Knekt *et al.*, 1990 (8)	Finland	Women	9	7.64/6.67 μg/dl	Serum	Four higher *vs.* lowest quintile	Urinary tract	2.51 (0.13–47.9)
Garland *et al.*, 1995 (12)	US	Women	28	0.826/0.812 μg/g	Toenail	Mean differences	Urinary tract	$P = 0.58$[a]
Present study	Netherlands	Both	431	0.540/0.561 μg/g	Toenail	Highest *vs.* lowest quintile	Urinary tract	0.67 (0.46–0.97)

[a] P for mean difference between cases and controls.

toenails reflect intake integrated for the previous 12 months or longer (12).

We were not able to explain our results on the basis of confounding because our results were essentially unchanged after incorporating into the analyses many known or suspected risk factors for bladder cancer, including current cigarette smoking; total consumption of alcohol, water, vegetables and fruit; high risk occupation; and positive family history of bladder cancer (data not shown). We made an attempt to model cigarette smoking habits such that they best explained bladder cancer. This resulted in a model including number of years smoked and habitual number of cigarettes smoked per day, both as continuous variables. When we added the smoking variables to an age- and sex-adjusted model, the RR estimates changed only slightly. We therefore believe that associations observed were not entirely due to residual confounding by smoking, although we cannot exclude some influence. It should be considered that we could not completely eliminate confounding bias because the inverse association found between low selenium levels and risk of bladder cancer may also indicate the protective effect of another nutrient in selenium-rich foods (26).

Recently, Cohen (29) suggested that transitional cell carcinoma of the urinary bladder might represent two distinct diseases: papillary transitional cell carcinoma, which is usually noninvasive, and the non-papillary type, which tends to be invasive. In addition to different prognostic and pathogenic properties, these two diseases appear to involve different molecular events (29). From an etiological point of view, some distinction might be important. An inverse association between toenail selenium level and bladder cancer risk was most pronounced in invasive transitional cell carcinomas of the urinary bladder, irrespective of tumor morphology. Further research is needed to evaluate this matter.

The association between total cancer incidence and selenium levels has been investigated in several prospective epidemiological studies. However, few results concerning urinary bladder cancer *per se* have been reported thus far (Refs. 7, 8, 12, and 13; Table 6). Although the number of bladder cancer cases in these studies was small, and the mean selenium levels differed substantially between the studies, most studies, as in the present study, demonstrated an inverse association between selenium concentrations and the risk of bladder cancer. Nomura *et al.* (13) reported a decrease in relative risk of bladder cancer for subjects in the highest quintile compared with the lowest quintile of serum selenium (RR = 0.32), although no statistically significant trend was found ($P = 0.12$). Helzlsouer *et al.* (7) similarly reported a decreased bladder cancer risk with increasing serum levels ($P = 0.03$). When examined by tertiles, the relative risk associated with the highest tertile of selenium compared with the lowest tertile was 0.49. A Finnish study (8) demonstrated a protective effect of selenium for men (RR = 0.34) but not for women (RR = 2.51) comparing the four highest quintiles against the lowest quintile of serum selenium, although the latter is based on only nine bladder cancer cases. Of interest in this connection is the fact that serum selenium levels in this Finnish study were approximately one-half of those found in the United States. Only one prospective study investigated the association between toenail selenium levels and female urinary tract cancer (12). This study did not find an association between toenail selenium and bladder cancer risk ($P = 0.58$; Ref. 12; Table 6).

Recently, Clark *et al.* (9) conducted a randomized, placebo-controlled cancer prevention trial in low-selenium areas of the United States to investigate whether selenium supplementation would decrease cancer incidence. The investigators demonstrated that, compared with controls, subjects treated with selenium had significant reductions in total cancer mortality (RR, 0.50; 95% CI, 0.31–0.80) and total cancer incidence (RR, 0.63; 95% CI, 0.47–0.85). For bladder cancer, the actual number of cases was too small to perform statistically meaningful analyses (9). Although promising, further human experimental research is needed to evaluate this matter.

The causal interpretation of an inverse association between selenium and cancer risk is supported by several animal experiments demonstrating anticarcinogenic effects of selenium, although bladder carcinogenesis has not often been investigated (30). Several hypotheses have been proposed to explain the inhibition of tumorigenesis by selenium, including alterations in carcinogen metabolism, effects on the endocrine and immune systems, production of cytotoxic selenium metabolites, inhibition of protein synthesis, inhibition of specific enzymes, inhibition of tumor growth, stimulation of apoptosis, and protection against oxidative stress involving the function of selenium as a constituent in the antioxidant enzyme glutathione peroxidase (7, 9–12, 26, 31, 32). The latter mechanism has received the most attention. The lack of effect of selenium status among nonsmokers is consistent with this hypothesis because never smokers have not been exposed to smoking-induced oxidative stress (33), although lack of effect among current smokers is not. It might be that the cumulative and continuing exposure to tobacco smoke among current smokers may overwhelm the effect of selenium. However, these differences might also be attributable the chance because no statistically significant effect modification was found.

We found evidence for a small inverse association between toenail selenium and bladder cancer risk. The decreased cancer incidence seems largely limited to the highest three quintiles of the toenail selenium distribution. The association was most pronounced in ex-smokers and was mainly confined to invasive carcinomas of the bladder. It appeared to be independent of β-carotene, vitamin C, and vitamin E intake.

Acknowledgments

We thank the regional cancer registries, the Dutch national database of pathology reports (PALGA), and the National Health Care Information Center for providing incidence data and S. Van de Crommert, H. Brants, W. van Dijk, M. Moll, J. Nelissen, C. de Zwart, A. Pisters, H. van Montfort, R. Schmeitz, T. van Montfort, T. van Moergastel, R. F. Marinus, and M. Sarilar for assistance.

References

1. Parkin, D. M., Pisani, P., and Ferlay, J. Estimates of the worldwide incidence of 25 major cancers in 1990. Int. J. Cancer, *80:* 827–841, 1999.

2. Silverman, D. T., Hartge, P., Morrison, A. S., and Devesa, S. S. Epidemiology of bladder cancer. Hematol. Oncol. Clin. N. Am., *6:* 1–30, 1992.

3. Johansson, S. L., and Cohen, S. M. Epidemiology and etiology of bladder cancer. Semin. Surg. Oncol., *13:* 291–298, 1997.

4. Ross, R. K., Jones, P. A., and Yu, M. C. Bladder cancer epidemiology and pathogenesis. Semin. Oncol., *23:* 536–545, 1996.

5. Bladder Cancer. *In*: Food, Nutrition and the Prevention of Cancer: A Global Perspective, pp. 338–361. Menasha (USA): Banta Book Group, 1997.

6. van der Meijden, A. P. M. Bladder cancer. Br. Med. J., *317:* 1366–1369, 1998.

7. Helzlsouer, K. J., Comstock, G. W., and Morris, J. S. Selenium, lycopene, α-tocopherol, β-carotene, retinol, and subsequent bladder cancer. Cancer Res., *49:* 6144–6148, 1989.

8. Knekt, P., Aromaa, A., Maatela, J., Alfthan, G., Aaran, R. K., Hakama, M., Hakulinen, T., Peto, R., and Teppo, L. Serum selenium and subsequent risk of cancer among Finnish men and women. J. Natl. Cancer Inst. (Bethesda), *82:* 864–868, 1990.

9. Clark, L. C., Combs, G. F., Jr., Turnbull, B. W., Slate, E. H., Chalker, D. K., Chow, J., Davis, L. S., Glover, R. A., Graham, G. F., Gross, E. G., Krongrad, A., Lesher, J. L., Jr., Park, H. K., Sanders, B. B., Jr., Smith, C. L., and Taylor, J. R. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin: a randomized controlled trial. J. Am. Med. Assoc., *276:* 1957–1963, 1996.

10. Fan, A. M., and Kizer, K. W. Selenium: nutritional, toxicologic and clinical aspects. West. J. Med., *153:* 160–167, 1990.

11. Virtamo, J., Valkeila, E., Alfthan, G., Punsar, S., Huttunen, J. K., and Karvonen, M. J. Serum selenium and risk of cancer. A prospective follow-up of nine years. Cancer (Phila.), *60:* 145–148, 1987.

12. Garland, M., Morris, J. S., Stampfer, M. J., Colditz, G. A., Spate, V. L., Baskett, C. K., Rosner, B., Speizer, F. E., Willett, W. C., and Hunter, D. J. Prospective study of toenail selenium and cancer among women. J. Natl. Cancer Inst. (Bethesda), *87:* 497–505, 1995.

13. Nomura, A., Heilbrun, L. K., Morris, J. S., and Stemmermann, G. N. Serum selenium and the risk of cancer, by specific sites: case-control analysis of prospective data. J. Natl. Cancer Inst. (Bethesda), *79:* 103–108, 1987.

14. Barlow, W. E., Ichikawa, L., Rosner, D., and Izumi, S. Analysis of case-cohort designs. J. Clin. Epidemiol., *52:* 1165–1172, 1999.

15. van den Brandt, P. A., Goldbohm, R. A., van het Veer, P., Volovics, A., Hermus, R. J. J., and Sturmans, F. A large-scale prospective cohort study on diet and cancer in the Netherlands. J. Clin. Epidemiol., *43:* 285–295, 1990.

16. van den Brandt, P. A., Schouten, L. J., Goldbohm, R. A., Dorant, E., and Hunen, P. H. M. Development of a record linkage protocol for use in the Dutch cancer registry for epidemiological research. Int. J. Epidemiol., *19:* 553–558, 1990.

17. Goldbohm, R. A., van den Brandt, P. A., and Dorant, E. Estimation of the coverage of Dutch municipalities by cancer registries and PALGA based on hospital discharge data. Tijdschr. Soc. Gezond., *72:* 80–84, 1994.

18. van den Brandt, P. A., Goldbohm, R. A., van't Veer, P., Bode, P., Hermus, R. J., and Sturmans, F. Predictors of toenail selenium levels in men and women. Cancer Epidemiol. Biomark. Prev., *2:* 107–112, 1993.

19. Bode, P., and de Bruin, M. An automated system for activation analysis with short half-life radionuclides using a carbon fiber irradiation facility. J. Radioanal. Chem., *132:* 365–375, 1987.

20. Spyrou, N. M. Activation analysis. J. Radioanal. Chem., *61:* 211–242, 1981.

21. Volovics, A., and van den Brandt, P. A. Methods for the analyses of case-cohort studies. Biom. J., *2:* 195–214, 1997.

22. Stata Statistical Software: Release 6.0. College Station, TX: Stata Corporation, 2000.

23. Barlow, W. E. Robust variance estimation for the case-cohort design. Biometrics, *50:* 1064–1072, 1994.

24. Lin, D. Y., and Ying, Z. Cox regression with incomplete covariate measurements. J. Am. Stat. Ass., *88:* 1341–1349, 1993.

25. Goldbohm, R. A., van den Brandt, P. A., Brants, H. A., van het Veer, P., Al, M., Sturmans, F., and Hermus, R. J. S. O. Validation of a dietary questionnaire used in large-scale prospective cohort study on diet and cancer. Eur. J. Clin. Nutr., *48:* 253–265, 1994.

26. Willett, W. C., Polk, B. F., Morris, J. S., Stampfer, M. J., Pressel, S., Rosner, B., Taylor, J. O., Schneider, K., and Hames, C. G. Prediagnostic serum selenium and risk of cancer. Lancet, *2:* 130–134, 1983.

27. van den Brandt, P. A., Goldbohm, R. A., van't Veer, P., Bode, P., Dorant, E., Hermus, R. J., and Sturmans, F. Toenail selenium levels and the risk of breast cancer. Am. J. Epidemiol., *140:* 20–26, 1994.

28. van den Brandt, P. A., van het Veer, P., and Goldbohm, R. A. A prospective cohort study on dietary fat and the risk of postmenopausal breast cancer. Cancer Res., *53:* 75–82, 1993.

29. Cohen, S. M. Urinary bladder carcinogenesis. Toxicol. Pathol., *26:* 121–127, 1998.

30. Mohandas, J., Marshall, J. J., Duggin, G. G., Horvath, J. S., and Tiller, D. J. Low activities of glutathione-related enzymes as factors in the genesis of urinary bladder cancer. Cancer Res., *44:* 5086–5091, 1984.

31. Patterson, R. E., White, E., Kristal, A. R., Neuhouser, M. L., and Potter, J. D. Vitamin supplements and cancer risk: the epidemiologic evidence. Cancer Causes Control, *8:* 786–802, 1997.

32. Salonen, J. T., Alfthan, G., Huttunen, J. K., and Puska, P. Association between serum selenium and the risk of cancer. Am. J. Epidemiol., *120:* 342–349, 1984.

33. van den Brandt, P. A., Goldbohm, R. A., van 't Veer, P., Bode, P., Dorant, E., Hermus, R. J., and Sturmans, F. A prospective cohort study on selenium status and the risk of lung cancer. Cancer Res., *53:* 4860–4865, 1993.